THE RELATION OF THE FIRST CLEAVAGE PLANE TO THE ENTRANCE POINT OF THE SPERM.*

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During the summer of 1911 at the Marine Biological Laboratory under the direction of Professor Frank R. Lillie, I was engaged in the study of the eggs of *Nereis* of certain cytological problems the results of which will appear later. The question of the relation of the entrance-point of the sperm and the first cleavage plane occurred to me. A very pretty method made possible in a satisfactory fashion the determination of this relation the results of which this paper embodies. I here take this opportunity to express my thanks and sense of gratitude to Professor Lillie for his inspiring interest in the work of which this is a part.

MATERIAL AND METHODS.

The eggs of *Nereis* when shed are irregular in shape due to pressure while in the body of the female. They soon fill out in the sea water, measuring about 100 μ equatorially and somewhat less in a polar direction. There is, however, a great deal of individual size variation in the eggs of a given female The eggs are almost transparent, colored a pale green by numerous deutoplasm spherules distributed throughout the endoplasm; around the equator is an irregular double girdle of 14 to 22 oil drops (Fig. 1). In polar view the large germinal vesicle appears to be in the center of the egg. It is, however, slightly elongated in the polar direction. The polarity of the ovocyte is, therefore, expressed by the polar flattening already mentioned, the position of the oil drops, and the form of the nucleus.

As has been shown (Lillie, '11) there are not two membranes in the unfertilized egg of *Nereis*, but rather a single vitelline membrane external to the radially striated cortical layer ("zona radiata," Wilson) of the egg. The ovocyte remains thus with

*All drawings, of living eggs, made with the aid of a camera lucida.

nucleus intact until inseminated or otherwise stimulated—as for instance, by squirting forcibly through a pipette.

Two or three minutes after insemination, a jelly is rhythmically extruded from the cortical protoplasm. In ten minutes the germinal vesicle breaks down, development is initiated.

Males and females captured in the evening while swimming at the surface of Eel Pond were kept in separate dishes until morning when they were transferred to fresh clean sea-water. To get an abundance of eggs and of sperm for an experiment, it was merely necessary to cut open a female and a male. The cut animals

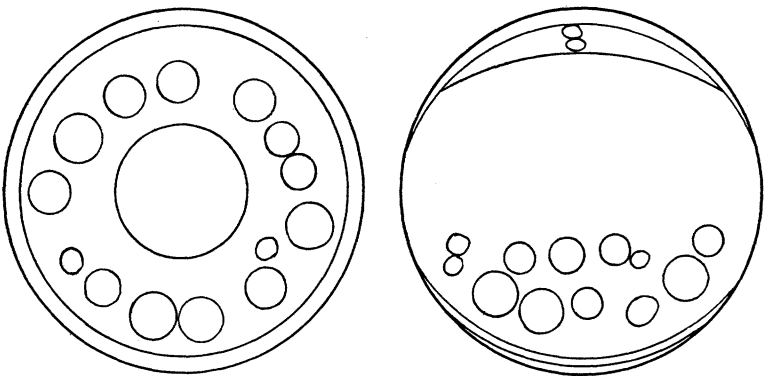

FIG. 1. Egg of *Nereis* at time of insemination; polar view.

FIG. 2. Maturation stage; second membrane formed; oil drops at vegetative pole.

were removed from the dishes at once; moreover, every other precaution was taken to avoid abnormalities superinduced through toxic influences, mechanical shock, etc. In several watch glasses of sea water in which India ink had been ground up eggs were put together with a minute quantity of sea water containing very few spermatozoa. The time of insemination was noted and the numbered dishes observed to the second cleavage. This method was varied somewhat as I shall later note.

Ringed slides also were used; eggs placed on these in sea water and ink were inseminated. Sometimes a cover slip was placed on the eggs. Finally, for the later observations a very few eggs were placed on slides and the cover slips supported with glass rods.

OBSERVATIONS.

Outline of Development to First Cleavage.

Eggs in sea-water in which India ink has been previously ground up show clearly the formation of the jelly, the formation of the fertilization cone, and the entrance of the spermatozoön. A streak of ink points like a dagger or an exclamation point to the entrance cone above which on the membrane the spermatozoön is attached (Fig. 4). This "exclamation point" is an aid quickly to determine in a large number of eggs the relation of the sperm entrance-point. The spermatozoön enters the egg at any point. (So also Lillie, '11.)

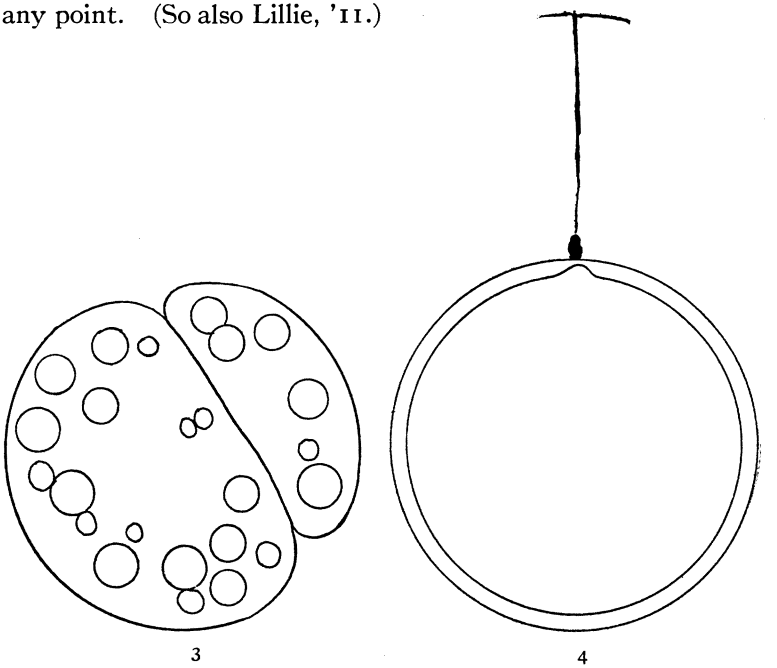

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FIG. 3. First cleavage.

FIG. 4. Cone and indicator formation, 15 minutes after insemination. Outer line marks boundary of jelly.

This ink "exclamation point," or "sperm indicator" as I shall call it, is a very interesting and striking formation worthy of detailed study. With me, however, the interest lay not so much in the formation of this indicator as in its availability to help answer the question: What is the relation of the sperm entrance

point to the first cleavage plane? I here, therefore, give only as much of an outline of its formation and of the development of the egg to the time of first cleavage as will suffice to render intelligible the subsequent record of observations.

Almost at the moment the spermatozoön touches the egg membrane, the contents of the cortical layer begin to flow out as a viscid transparent substance of the same refractive index as water, leaving only radiating lines across the space (perivitelline space) between protoplasm and membrane which represent the walls of the emptied alveoli. This jelly in its flow carries the ink from the periphery of the egg so that between each egg and the surrounding ink is a clear space. This outflow of jelly may last for fifteen minutes. The jelly forms about the egg a layer everywhere continuous except along the tail of the sperm which thus forms a canal that increases in length as the jelly area widens.

Below the spermatozoön, the protoplasm of the egg begins to form a cone at thirteen to fifteen minutes after insemination which gradually increases in height until it reaches the membrane and then slowly retrogresses. With this retrogression, the membrane at this point sinks; in this depression lies the sperm. During this behavior, as the jelly area widens, the canal in the jelly in which the tail of the sperm lies fills in with particles of ink. This process is a gradual one, the indicator reaching its maximum of development fifteen to twenty minutes after insemination. The indicator, therefore, is formed along the tail of the sperm and points to the entrance-point of the sperm.

Twenty minutes after insemination, the spermatozoön may be seen attached to the membrane at the end of the indicator. The perivitelline space now becomes slight. The egg "assumes an amœboid appearance" (Wilson), changing its shape and becoming very irregular. The sperm cannot be seen readily (Fig. 5). About forty minutes after insemination the egg becomes spherical again. The sperm is easily visible on the membrane which is more widely separated from the protoplasm by the perivitelline space.

This condition is of short duration for the egg begins another series of changes. The membrane appears everywhere equi-

distant from the egg except at the point of sperm attachment where it is nearer the membrane. Then gradually to the right and left of the point of sperm attachment the perivitelline space becomes greater; the egg elongates along a line through the point of sperm attachment (Fig. 6). With the disappearance of the sperm head within the egg (about fifty minutes after in-

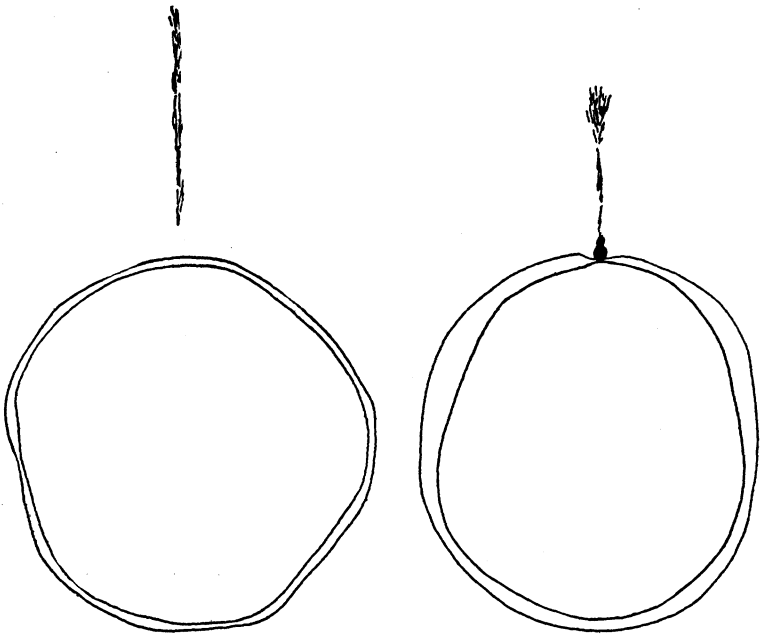

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FIG. 5. After retraction of cone; membrane close to the egg. Sperm apparently in the egg.

FIG. 6. Two minutes before sperm is engulfed.

semination) this elongated appearance is lost (Fig. 7): the egg rounds out. The egg flattens at the animal pole (Fig. 8) and the polar bodies are given off from a clear apparently yolk-free region of the flattened pole (Fig. 2). Some little time later the first cleavage furrow appears and the egg is divided unequally (Fig. 3).

The observations on the relation of this cleavage to the entrance-point of the sperm will be considered under three heads corresponding to the methods used.

Watch Glass Series.

A female was opened at 9:58, a male at 10:00. In five watch glasses of india ink ground up in sea-water eggs and sperm were mixed at intervals of two minutes. At 10:10 a few eggs were

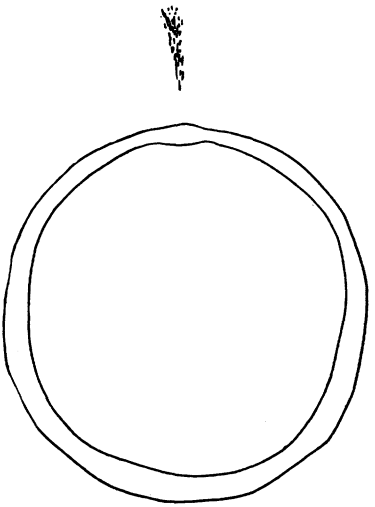

FIG. 7. Just after disappearance of sperm within the egg.

inseminated in the ink solution on an uncovered slide (no. 6). About two minutes after an insemination the jelly began to form; in fifteen minutes the sperm indicator was well developed. Eggs

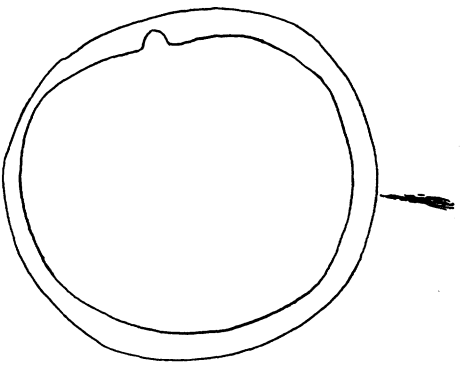

FIG. 8. First polar body forming.

inseminated at 10:15 in a watch glass (no. 7) were washed at 10:30: that is, when the indicator had reached its maximum of development.

The dishes (nos. 1 to 5) and the slide (no. 6) were examined as the first cleavage furrow appeared. In 95 per cent. of the eggs the first cleavage plane passed through the point of sperm entrance (Fig. 9). Dish no. 7 showed, on the other hand, that in only 50 per cent. of the eggs the first cleavage furrow passed through the point of sperm entrance.

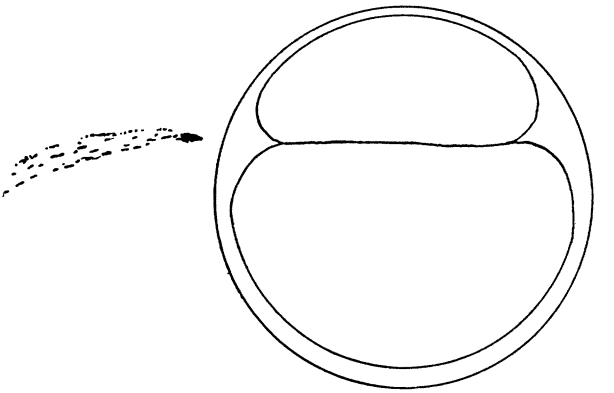

FIG. 9. First cleavage.

At 2:45 p.m. of the same day, eggs were inseminated in watch glass no. 8. Examination revealed that the first cleavage plane passed through the point of entrance in 80 per cent. of eggs. Eggs transferred from india ink and sea-water to clean fresh sea-water twenty to thirty minutes after insemination showed 60 per cent. of first cleavages through the point of entrance.

A summary of the results of Experiments 1 to 8 is as follows:

Nos. 1–5 inseminated in watch glass, not washed, showed first cleavage through entrance point, 95 per cent.

No. 6 inseminated on slide glass, not washed, showed first cleavage through entrance point, 95 per cent.

No. 7 inseminated in watch glass, washed, showed first cleavage through entrance point, 50 per cent.

No. 8 inseminated in watch glass, not washed, showed first cleavage through entrance point, 80 per cent.

No. 9 inseminated in watch glass, transferred to slide, showed first cleavage through entrance point, 60 per cent.

That the ink is not toxic to the eggs and, therefore, does not inhibit cleavage I was able to prove by inseminating at the same time two dishes of eggs, one with ink and one without; develop-

ment in both went on at the same rate and in perfectly normal fashion. I concluded, therefore, that it was not necessary to wash the eggs. Also, I found later that the eggs were often too greatly crowded and that it was hard to make counts unless the eggs were in a single layer. A trial made with very few eggs unwashed in four watch glasses gave the following result (actual numbers are given):

FIRST CLEAVAGE PLANE.

Number.	Through Entrance Point.	Not Through Entrance Point.
1	8	2
2	16	4
3	10	1
4	12	3

To what extent the eggs might rotate in the jelly was yet to be determined. It was absolutely necessary that the relation of the indicator and the sperm entrance-point remain constant; otherwise, the indicator would prove a very pretty but useless phenomenon. Could it be possible for two spermatozoa to reach the egg and the indicator to form along one sperm and not the other? How would such an egg cleave? These points were next to be determined.

I found, first, that the position of the indicator could be altered through tilting the watch glass, for the eggs would rotate in the jelly—especially when they lay on the side. I found later that the eggs are most liable to rotation after the sperm has disappeared. This might easily prove a serious source of error. Secondly, I demonstrated in several experiments that polyspermic eggs are not apt to cleave. (Professor Lillie has obtained the same results.) But with fairly dilute sperm and sea water, polyspermy, which merely cuts down the number of cleaving eggs, may be avoided.

In this connection it will be interesting to note the results obtained with old eggs and sperm. On July 30 eggs from a female captured in the evening of July 28 were used with fresh sperm—of a male captured in the evening of July 29. These eggs proved very susceptible to polyspermy. This proved true in other trials. These eggs if they segmented at all showed sixty per cent. of first cleavages through the entrance-point of the

sperm. In general, eggs that have stood in sea water for some time after leaving the female, show a low per cent. of cleavages through the entrance-point. Five hours after leaving the female eggs fail to develop on insemination.

These results seem to indicate that the first cleavage tends to pass through the sperm entrance point—*i. e.*, through the point at the end of the indicator—if the eggs be fresh, undisturbed and fertilized with a single sperm. Why then do some first cleavages fail to pass through this point? During this time a number of experiments made by day and often at night immediately after the capture of the animals showed essentially the same proportions.

Ringed Slide Series.

It was stated above, it will be remembered, that the egg tends to lie with either pole uppermost. If, however, the eggs are not disturbed those that settle on the side will so remain. The eggs are accessible to sperm at any point if not under pressure as at no time in this study they were. The first cleavage always cuts through the animal pole near the polar bodies. Obviously then, the question of the relation of the first cleavage plane to the entrance-spot of the sperm cannot be settled by the cleavage of those eggs in which the spermatozoa enter either at the point below which the polar bodies are extruded or at a spot 180° from this point.

In the next trial with very few eggs on ringed slides, those eggs in which the sperm indicator pointed either to the polar bodies or to a point 180° from the polar bodies were not counted. This trial resulted as follows:

FIRST CLEAVAGE PLANE.

Number.	Through Entrance Point.	Not Through Entrance Point.
1	14	4
2	6	4
3	20	9

Other experiments with ringed slides showed about the same proportions.

For fear that the ringed slides were toxic owing to the vaseline used they were abandoned and slides with cover slips supported

by glass rods as well as the open watch glass were used throughout the next series of observations.

Slides with Glass Support for Cover Slip.

Four or five eggs on a slide were watched continuously through the first cleavage, the indicator used merely to point out quickly the point on the membrane where the sperm was attached. Very few sperm were used in these observations, obtained through diluting several times the water which contained them. These observations were repeatedly made at night and at different times during the day. Some of the eggs failed to show the indicator and to develop. In all that segmented, *the first cleavage plane passed either directly through the entrance-point of the sperm*

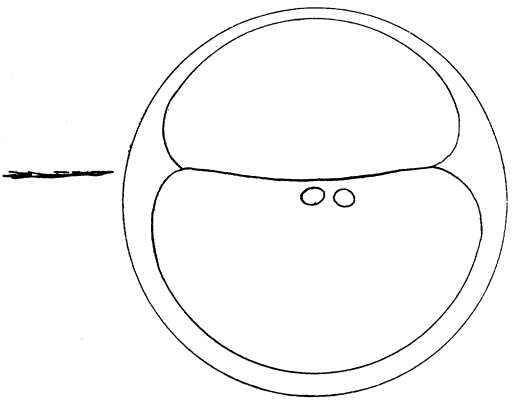

FIG. 10. First cleavage.

or a degree or so from it, with the indicator parallel to the cleavage furrow (Fig. 10). It is possible, as stated above, to keep the spermatozoön in view after the amœboid stage until it disappears within the egg. The middle piece is left without. With the aid of the middle piece, the character of the membrane at the entrance point (Fig. 7), and the oil drops near, it is possible absolutely to hold in view the exact spot at which the sperm was engulfed.

At intervals of two to three minutes, seven slides with very few eggs on each were prepared. Sperm was added and after a minute the eggs covered and every precaution taken to avoid

disturbance. In the sixty eggs counted the first cleavage furrow passed through the sperm entrance-point in every case. In some cases the indicator appeared to be at right angles to the furrow but in all such it proved to be *above* the egg and ended in the cleavage plane (Fig. 11). This was Sunday, August 20. The laboratory was quiet, the temperature conditions favorable. The results of August 23, 24 and 27 are similar. Camera sketches

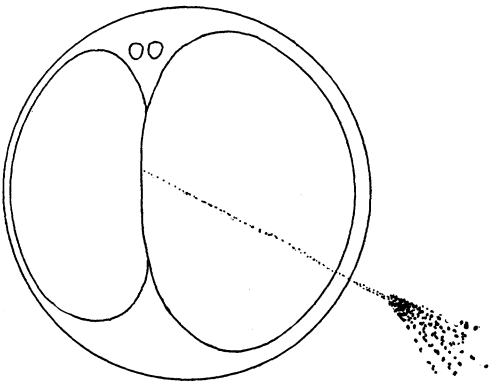

FIG. 11. First cleavage.

were made of these eggs. Often I asked an investigator, who did not know the purport of the experiments, to make the sketches; the indicator without doubt was above the egg and pointed to the cleavage furrow.

DISCUSSION.

The first cleavage plane usually coincides with the median plane of the future animal in the frog's egg, according to Roux, Newport, Pflüger and Morgan. In the squid's egg also, according to Watasé, the first cleavage plane falls in with the median plane of the embryo. Agassiz and Whitman ('84) noted a like coincidence in the teleost egg; and Van Beneden and Julin, Castle ('96) and Conklin ('05) found that the first cleavage plane marks the long axis of the embryo in the ascidian egg.[1]

[1] According to Harper, the sperm enters the pigeon's egg previous to the egg's entrance into the oviduct. He believes that the sperm must enter as soon as the disc is exposed through rupture of the follicular wall. In the pigeon's egg the sperm entrance is more or less localized. According to his figure, the first cleavage plane makes an angle of 45° with the long axis of the embryo. As we know from other researches, the long axis of the embryo is similarly placed in the egg.

But there are other eggs in which the future median plane does not fall in the plane of the first cleavage. In *Nereis* (Wilson, '92) the *second* cleavage plane, although it does not divide the animal into "equal halves," coincides with the long axis. So in *Crepidula*, the first cleavage plane is at right angles to the future median plane (Conklin, '97). In the newt (Jordan, '93) the case is the same. In *Chætopterus* (Lillie, '06) the axis of the first cleavage spindle lies in the longitudinal axis of the embryo.

There is a third group of eggs in which coincidence with any cleavage plane is wanting This is true of the egg of *Amia* (Whitman and Eycleshymer, '97), of the toadfish (Clapp, '91), and of certain amphibians (Jordan and Eycleshymer, '94), to name a few. And yet in most of these eggs the symmetry and the bilaterality of the cleavage may be sharply marked.

In the frog's egg the first cleavage plane usually and the median plane of the embryo always (*Rana fusca*) pass through the entrance point of the sperm (Roux, '85; Schulze, '99; Brachet).

In the egg of *Toxopneustes* (Wilson, '95) the first cleavage plane passes through the entrance-point of the sperm, "in the great majority of cases, at least." This plane of cleavage coincides with the transverse diameter of the embryo (Driesch).

In the ascidian egg, the belief of Castle ('96) is that the first cleavage plane cuts through the entrance-point of the sperm. Conklin ('05) says that there is no question but that the first cleavage plane is through the copulation path of the germ nuclei. And indeed his figures show very beautifully that this is actually the case.

If now we grant that in the egg of the frog and of *Toxopneustes* as in the egg of *Nereis* and of the ascidian the first cleavage plane is determined by the copulation-path, or the entrance-point, of the sperm we have this interesting conclusion: The first cleavage plane in eggs whose cleavages have different values and different relations to the future long axes of the embryos is determined by the entrance of the sperm. While the sperm entrance determines the first cleavage, the first cleavage does not in all of these forms coincide with the median plane of the future animal.

Since in the egg of *Nereis* the sperm may enter at any point and since the first cleavage plane passes through this point, the struc-

ture of the ovocyte of *Nereis at the time of insemination* must be the same in all meridians. This, I believe, has an important bearing on theories of germinal areas in the cytoplasm, of prelocalization, and of precocious segregation. The determination of bilaterality follows fertilization.

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